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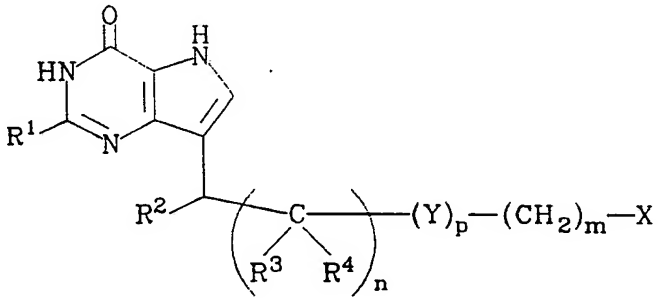

(I)

**(57) Abstract**

Disclosed is a compound of formula (I) where $R^1$ is H, $NH_2$, or $OCH_3$, $R^2$ is an optionally substituted cyclic group optionally containing one or more heteroatoms, $R^3$ and $R^4$ are independently H or $C_{1-4}$ alkyl, m is 0-4, n is 0-6, p is 0.1, X is CN, $CSNH_2$, $PO(OH)_2$, COOH, $SO_2NH_2$, $NH_2$, OH, $CNHNH_2$, tetrazole, triazole, or $COR^5$ where $R^5$ is $C_{1-4}$ alkyl, $CF_3$, $NH_2$, or $OC_{1-4}$ alkyl, and Y is O or NH that is useful as a pharmaceutical.

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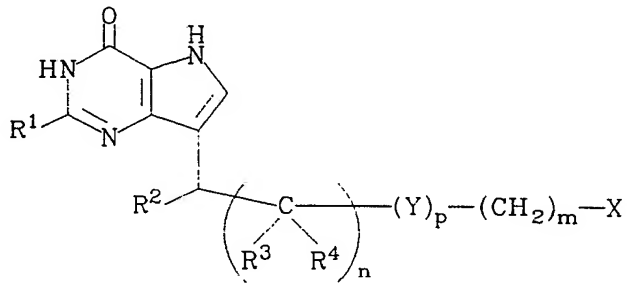
# 7-DISUBSTITUTED-METHYL-4-OXO-3H,5H-PYRROLO[3,2-d]PYRIMIDINE AND PHARMACEUTICAL USES AND COMPOSITIONS CONTAINING THE SAME

The present invention relates to derivatives of 4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidine. In particular, it relates to 4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidine derivatives substituted at the 7-position.

Purine nucleoside phosphorylase (PNP) catalyzes the phosphorolysis of purine nucleosides in a reversible reaction. Individuals who are deficient in PNP exhibit impaired T-cell development, resulting in lowered cell-mediated immunity, but normal B-cell development, resulting in normal humoral immunity. Accordingly, specific inhibitors of PNP that selectively inhibit T-cell development without damaging humoral immunity could be potentially effective against disorders in which activated T-cells are pathogenic.

Accordingly, the present invention is a compound of the formula



wherein $R^1$ is H, $NH_2$, or $OCH_3$, $R^2$ is an optionally substituted cyclic group optionally containing one or more heteroatoms, $R^3$ and $R^4$ are independently H or $C_{1-4}$ alkyl, m is 0-4, n is 0-6, p is 0-1, X is CN, $CSNH_2$, $PO(OH)_2$, COOH, $SO_2NH_2$, $NH_2$, OH, $CNHNH_2$, tetrazole, triazole or $COR^5$ where $R^5$ is $C_{1-4}$ alkyl, $CF_3$, $NH_2$, or $OC_{1-4}$ alkyl, and Y is O or NH. The compound of the present invention is useful as a PNP inhibitor. Also contemplated according to the present invention are a pharmaceutical composition for the selective suppression of mammalian T-cell immunity comprising an pharmaceutically effective amount of the compound of the present invention and a pharmaceutically acceptable carrier or diluent and a method for the selective

suppression of mammalian T-cell immunity without diminished effect on humoral immunity comprising administering to a subject a pharmaceutically effective amount of the compound of the present invention.

The optionally substituted cyclic group (hereinafter referred to as cyclo) recited for the above formula includes aromatic, heteroaromatic, alicyclic, and heteroalicyclic groups preferably containing five to nine atoms. Preferred optional substituents include halogen, hydroxy, alkoxy, alkyl, and trifluoromethyl. Exemplary substituents include chloro, fluoro, methoxy, ethoxy, propoxy, butoxy, methyl, ethyl, propyl, and butyl. Preferred heteroatoms include oxygen, nitrogen, and sulfur, which can be present in combination in the same group. The preferred aromatic and heteroaromatic groups are phenyl, 2- or 3-thienyl, 2- or 3-furanyl, 2-, 3-, or 4-pyridinyl, 2- or 3-pyrrolyl, 2-, 4-, or 5-thiazolyl, 2-pyrazinyl, 3- or 4-pyridazinyl, and 3-, 4-, or 5-pyrazolyl. The preferred alicyclic and heteroalicyclic groups are 1- or 2-adamantyl, cyclohexyl, cycloheptyl, 2- or 3-tetrahydrofuranyl, 2- or 3-tetrahydrothienyl, 2- or 3-tetrahydropyranyl, 2-, 3-, or 4-piperidinyl, 3- or 4-pyrazolidinyl, 2-, 4-, or 5-thiazolidinyl, 2- or 3-piperazinyl, 2- or 3-morpholinyl, or 3- or 4-hexahydropyridazinyl. Examples include compounds wherein $R^1$ is $NH_2$ or H, $R^2$ is phenyl, 3-chlorophenyl, or 3,4-dicholorophenyl, and $(CR^3R^4)_n-(Y)_p-(CH_2)_m-X$ is $CH_2CH_2CN$; $CH_2CH_2COOH$; $CH_2CH_2CH_2OH$; $CH_2CH_2CH_2CN$; $CH_2CH_2CH_2COOH$; $CH_2CH_2CH_2CH_2OH$, or substituents where an oxygen atom replaces one or more of the methylene groups.

The present invention contemplates pharmaceutical compositions suitable for enteral, such as oral or rectal, transdermal and parenteral administration to mammals including man, which are useful to inhibit purine nucleoside phosphorylase activity and for the treatment of disorders responsive thereto, comprising an effective amount of a pharmacologically active compound of the invention, alone or in combination, with one or more pharmaceutically acceptable carriers.

Preferred pharmaceutical compositions are tablets and gelatin capsules comprising the active ingredient together with

a) diluents, e.g., lactose, dextrose, sucrose, mannitol, sorbitol, cellulose and/or glycine; b) lubricants, e.g., silica, talcum, stearic acid, its magnesium or calcium salt and/or polyethyleneglycol; for tablets also c) binders, e.g., magnesium aluminum silicate, starch paste, gelatin, tragacanth, methylcellulose, sodium carboxymethylcellulose and/or polyvinylpyrrolidone; if desired d) disintegrants, e.g., starches, agar, alginic acid or its sodium salt, or effervescent mixtures; and/or e) absorbents, colorants, flavors and sweeteners. Injectable compositions are preferably aqueous isotonic solutions or suspensions, and suppositories are advantageously prepared from fatty emulsions or suspensions. Said compositions may be sterilized and/or contain adjuvants, such as preserving, stabilizing, wetting or emulsifying agents, solution promoters, salts for regulating the osmotic pressure and/or buffers. In addition,they may also contain other therapeutically valuable substances. Said compositions are prepared according to conventional mixing, granulating or coating methods, respectively, and contain about 0.1 to 75%, preferably about 1 to 50%, of the active ingredient.

Suitable formulations for transdermal application include an effective amount of a compound of the invention with a carrier. Advantageous carriers include absorbable pharmacologically acceptable solvents to assist passage through the skin of the host. Characteristically, transdermal devices are in the form of a bandage comprising a backing member, a reservoir containing the compound optionally with carriers, optionally a rate controlling barrier to deliver the compound to the skin of the host at a controlled and predetermined rate over a prolonged period of time, and means to secure the device to the skin.

Another aspect of the present invention provides a method of making a 2-amino compound ($R^1 = NH_2$) of the present invention and intermediates thereof. The first step of the method involves reacting an optionally substituted cyclic aldehyde with cyanoacetic acid at a molar ratio of about 1/1 to 1/5 in the presence of ammonium acetate at about reflux temperature for

about 10 hours to 8 days to make a 3-cyclo-substituted pentanedinitrile as an intermediate. In the second step, the 3-cyclo-pentanedinitrile is reacted with an alkyl formate such as ethyl formate and a strong base such as the metal-containing bases sodium hydride or sodium alkoxide, e.g., sodium methoxide, at a molar ratio of about 1-2/3-6/1-3 and at a temperature of about 20-65°C for about 10 hours to 8 days to make a 3-cyclo-2-formylpentanedinitrile as a further intermediate. The next step involves reacting the 3-cyclo-2-formylpentanedinitrile with a glycine alkyl ester hydrochloride and sodium or ammonium acetate at a molar ratio of about 1-2/1.5-4/1.5-4 and at a temperature of about 20-60°C for about 10-48 hours to make methyl N-[(3-cyclo-2,4-dicyano)-2-butenyl]glycine as an intermediate. In the subsequent step, the methyl N-[(3-cyclo-2,4-dicyano)-2-butenyl]glycine is reacted with an alkyl chloroformate such as ethyl chloroformate and 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) or 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) at a molar ratio of about 1-2/1.5-5/1.5-4 and at a temperature of about 0-50°C for about 10 hours to 10 days to make methyl 3-amino-4-(2-cyano-1-cyclo-ethyl)-1-ethyl-1H-pyrrole-1,2-dicarboxylate as an intermediate. The next step involves reacting the methyl 3-amino-4-(2-cyano-1-cyclo-ethyl)-1-ethyl-1H-pyrrole-1,2-dicarboxylate with a base such as sodium carbonate at a molar ratio of about 2/1 to 1/5 and at about room temperature for about 10-48 hours to make methyl 3-amino-4-(2-cyano-1-cyclo-ethyl)-1H-pyrrole-2-carboxylate as an intermediate. In the next step, the methyl 3-amino-4-(2-cyano-1-cyclo-ethyl)-1H-pyrrole-2-carboxylate is reacted with benzoylisothiocyanate at a molar ratio of about 2/1 to 1/2 and at about room temperature for about 30 minutes to 3 hours to make N-benzoyl-N'-[4-(2-cyano-1-cyclo-ethyl)-2-methoxycarbonyl-1H-pyrrol-3-yl]thiourea as an intermediate. The next step reacts the N-benzoyl-N'-[4-(2-cyano-1-cyclo-ethyl)-2-methoxycarbonyl-1H-pyrrol-4-3-yl]thiourea with an alkyl halide such as methyl iodide at a molar ratio of about 1/1 to 1/6 and at a temperature of about 0-30°C for about 10 minutes to 10 hours to make N-benzoyl-N'-[4-(2-cyano-1-cyclo-ethyl)-2-methoxycarbonyl-1H-pyrrol-3-yl]S-methylthiourea as an

intermediate. In the following step, the N-benzoyl-N'-[4-(2-cyano-1-cyclo-ethyl)-2-methoxycarbonyl-1H-pyrrol-3-yl]-S-methylthiourea (about 1-2 mol) is reacted with methanolic or ethanolic ammonia at a ratio of about 1/1 to 1/20 and at a temperature of about 20-130°C for about 16-60 hours to make a mixture of a 2-amino compound of the present invention 3-cyclo-3-[2-amino-4-oxo-3H-5H-pyrrolo[3,2-d]pyrimidin-7-yl]propanenitrile and a 3-cyclo-3-[2-methylmercapto-4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidin-7-yl]propanenitrile as an intermediate in making another compound of the present invention.

In a further aspect of the present invention there is provided a method of making a 2-methoxy compound ($R^1$ = $OCH_3$) and intermediates thereof. The intermediate 3-cyclo-3-[2-methylmercapto-4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidin-7-yl]propanenitrile is reacted with an oxidizing agent such as permanganate or hydrogen peroxide at a molar ratio of about 1/1 to 1/10 and at a temperature of about 25-120°C for about 3-48 hours to make 3-cyclo-3-[2-methylsulfonyl-4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidin-7-yl]propanenitrile as an intermediate. In the next step, the 3-cyclo-3-[2-methylsulfonyl-4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidin-7-yl]propanenitrile is reacted with a sodium alkoxide such as sodium methoxide at a molar ratio of about 1/1 to 1/10 and at a temperature of about 25-100°C for about 1-48 hours to make a 2-methoxy compound of the present invention 3-cyclo-3-[2-methoxy-4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidin-7-yl]propanenitrile.

In a further aspect of the present invention there is provided a method of making a compound of the present invention wherein $R^1$ is hydrogen. The methyl 3-amino-4-(2-cyano-1-cyclo-ethyl)-1H-pyrrole-2-carboxylate intermediate described supra is reacted with dimethylformamide dimethyl acetal at a molar ratio of about 1/1 to 1/4 and at a temperature of about 25-100°C for about 1-10 days to make methyl 4-(2-cyano-1-cyclo-ethyl)-3-[N-(dimethylaminomethylene)amino]-1H-pyrrole-2-carboxylate as an intermediate. The next step involves reacting the methyl 4-(2-cyano-1-cyclo-ethyl)-3-[N-(dimethylaminomethylene)amino]-1H-pyrrole-2-carboxylate with methanolic or ethanolic ammonia at a

molar ratio of about 1/1 to 1/20 and at a temperature of about 20-130°C for about 10-68 hours to make the compound of the present invention 3-cyclo-3-[4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidin-7-yl]propanenitrile.

As will be apparent to the skilled artisan, variations of the aforesaid procedures are useful in making the variety of compounds of the present invention without departing from the spirit thereof. For example, compounds having different values for "n" and "m" in accordance with the present invention are obtained by either stepping up or stepping down the series by the necessary number of carbon atoms in accordance with known procedures. Also, reactions involving some intermediates require protection of nitrogen or oxygen atoms on the intermediates using known procedures.

The present invention provides a method of inhibiting purine nucleoside phosphorylase activity in mammals and treating diseases and conditions responsive thereto, e.g., autoimmune disorders, rejection of transplantation, or psoriasis, which comprises administering to a mammal in need thereof an effective amount of a compound of the invention or of a pharmaceutical composition comprising a said compound in combination with one or more pharmaceutically acceptable carriers.

A further aspect of the invention relates to a method of inhibiting the phosphorolysis and metabolic breakdown of antiviral or antitumor purine nucleosides in mammals which comprises administering in conjunction therewith to a mammal in need thereof, either separately or in combination therewith, an effective purine nucleoside phosphorylase inhibiting amount of a compound of the invention or of a said compound in combination with one or more pharmaceutically acceptable carriers. More particularly, such relates to a method of inhibiting the phosphorolysis and metabolic breakdown of purine nucleosides known in the art, e.g., of 2'-deoxyguanosine, 2',3'-dideoxyinosine, 2',3'-dideoxyguanosine or 2',3'-dideoxyadenosine.

Furthermore, the invention thus relates to a method of potentiating the antiviral or antitumor effect of 2' or 3'-monodeoxypurine nucleosides or of 2',3'-dideoxypurine nucleosides

in mammals which comprises administering in conjunction therewith to a mammal in need thereof, either separately or in combination with a said nucleoside, an effective purine nucleoside phosphorylase inhibiting amount of a compound of the invention preferably in combination with one or more pharmaceutically acceptable carriers. More particularly, such relates to a method of enhancing or potentiating the effect of 2',3'-dideoxypurine nucleosides known in the art, e.g., of 2',3'-dideoxyinosine, 2',3'-dideoxyguanosine or 2'-3'-dideoxyadenosine for the treatment of retrovirus infections, e.g., HIV-retrovirus infections (acquired immunodeficiency syndrome, AIDS). 2',3'-Dideoxypurine nucleosides are known in the art as inhibitors of HIV retrovirus infectivity and to be metabolically degraded by PNP, e.g., as described in Biochemical Pharmacology 22, 3797 (1987). Such are administered at a pharmaceutically acceptable dose which is effective in inhibiting HIV-retrovirus infections. Preferably the lowest possible effective dose is used.

The pharmaceutically acceptable effective dosage of active compound of the invention to be administered is dependent on the species of warm-blooded animal (mammal), the body weight, age and individual condition, and on the form of administration.

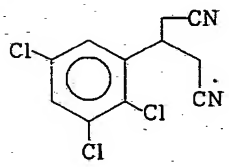
The pharmaceutical composition may be oral, parenteral, suppository or other form which delivers the compound of the present invention into the bloodstream of a mammal to be treated. An oral form has from about 1 to about 150 mg of the compound of the present invention for an adult (50 to 70 kg) which is mixed together with pharmaceutically acceptable diluents such as lactose. In a typical capsule, 25 mg of the compound of the present invention is mixed together with 192 mg lactose, 80 mg modified starch and 3 mg magnesium stearate. Injectable forms of the compound are also contemplated for administration.

The present invention is also useful with other therapeutic agents. A daily dosage of the compound of the present invention for a human weighing 50 to 70 kg of 1-50 mg/kg inhibits metabolic destruction of certain anticancer agents such as $\beta$-2'-deoxy-6-thioguanosine and antiviral agents such as 2',3'-dideoxyinosine, an anti-AIDS drug. These types of agents are known to be

susceptible to cleavage. Upon cleavage, the agents lose effectiveness. The compounds of the present invention are capable of reducing such cleavage. This protection, therefore, enhances the efficacy of other chemotherapeutic agents.

In order to more fully describe the present invention the following non-limiting examples are provided. In the examples all parts and percentages are by weight unless indicated otherwise. Proportions of solvent mixtures used as chromatographic eluents are by volume.

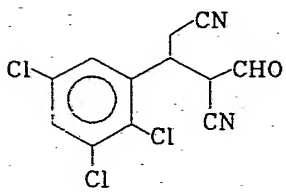
**EXAMPLE 1**




The above intermediate compound is prepared in this Example by the modification of the procedure of Schiemenz, G. P.; Engelhard, H. (Chem. Ber., **1962**, 95, 195).

A mixture of cyanoacetic acid (25.38 g, 298.38 mmol), 2,3,5-trichlorobenzaldehyde (25.0 g, 119.35 mmol), ammonium acetate (500 mg), toluene (120 ml), and pyridine (65 ml) is heated at reflux for 16 h in a flask fitted with Dean-Stark trap and condenser. The solvents are evaporated in vacuo, residue is extracted with $CHCl_3$, which is washed with $H_2O$, dried ($Na_2SO_4$), and evaporated to give the crude product, which is purified by silica gel column chromatography using hexane-EtOAc mixture as the eluent. Yield 23.69 g (73%); mp 90-91 °C.

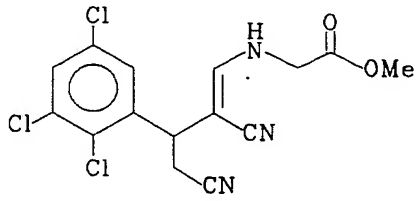
**EXAMPLE 2**




The above intermediate compound is prepared in this Example. To a stirred mixture of NaH (1.56 g, 65.05 mmol) and ethyl

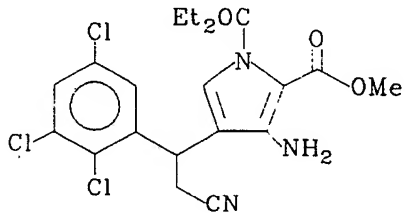
formate (14.78 g, 199.51 mmol) in THF (100 ml) is added substituted pentanedinitrile of Example 1 (10.17 g, 37.17 mmol) at room temperature under a nitrogen atmosphere, and the resulting reaction mixture is stirred for 24 h. Volatile matter is evaporated in vacuo at room temperature. Water (50 ml) is added to the residue at 0-5 °C, and the solution is adjusted to pH 5-6 by 20% conc. HCl (v/v). The heavy oil is extracted into ethyl acetate, washed with $H_2O$ (1 x 100 ml) and dried ($MgSO_4$). The ethyl acetate layer is evaporated to give a red-brown oil (11.0 g) that is used in the next step without further purification.

**EXAMPLE 3**



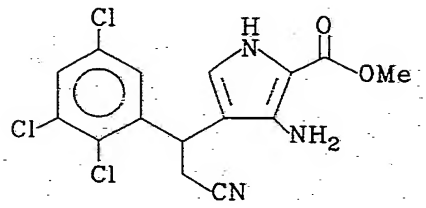

The above intermediate compound is prepared in this Example. Glycine methyl ester hydrochloride (8.17 g, 65.06 mmol) and sodium acetate (5.33 g, 65.06 mmol) are added to a solution of the crude formyl compound of Example 2 (11.0 g) in a mixture of MeOH (80 ml) and $H_2O$ (20 ml), and the resulting solution is stirred at room temperature for 22 h. After evaporation of solvent at room temperature, the residue is extracted with ethyl acetate. The washed ($H_2O$) and dried ($MgSO_4$) organic layer is evaporated to give an oil. Flash column chromatography (silica gel) using $CHCl_3$ as eluent gave the pure desired enamine as a mixture of cis-trans isomers which is recrystallized from MeOH, yield 10.41 g (75%), mp 142-143 °C.

**EXAMPLE 4**



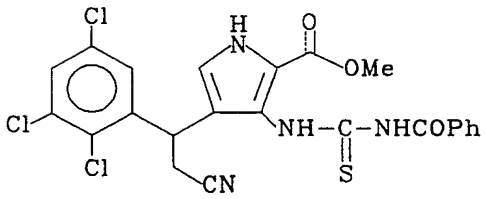
The above intermediate compound is prepared in this Example. A solution of enamine of Example 3 (10.0 g, 26.84 mmol) in dry $CH_2Cl_2$ (100 ml) is cooled to 0 °C and treated with 1,5-diazabicyclo[4.3.0]non-5-ene (10.53 g, 84.79 mmol) under a nitrogen atmosphere followed by ethyl chloroformate (6.90 g, 63.57 mmol). The solution is stirred at 0 °C for 1 h and then at room temperature for 48 h. Volatiles are evaporated <u>in vacuo</u> to give a viscous dark gum which is purified by flash column chromatography over silica gel using $CHCl_3$ as the eluent. All the fractions containing the desired N-protected pyrrole are pooled and evaporated to give a foamy light pale yellow material which is stirred in MeOH (100 ml) to give the crystalline material which is recrystallized from $CHCl_3$-MeOH, yield 8.92 g (74.7%), mp 160-161 °C.

**EXAMPLE 5**



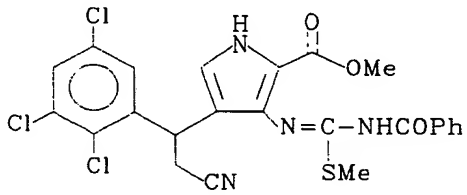

The above intermediate compound is prepared in this Example. A suspension of N-protected pyrrole of Example 4 (8.6 g, 19.34 mmol) in MeOH (300 ml) is treated with $Na_2CO_3$ (5.12 g, 48.34 mmol) and the reaction mixture is stirred at room temperature for 17 h with separation of the deblocked pyrrole during the first hour. Solid sodium carbonate is removed by filtration and washed well with MeOH. The filtrate is reduced to a volume of ~25 ml and kept in a refrigerator for 1 h to give 5.23 g of crystalline product. Further concentration of the mother liquor gave an additional 0.14 g of pure product; total yield 6.45 g (89.5 %), mp 178-181 °C.

**EXAMPLE 6**



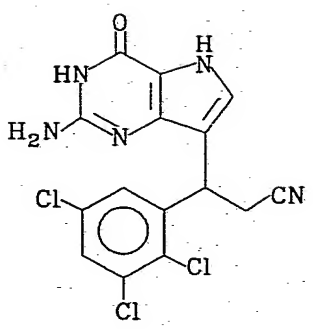
The above intermediate compound is prepared in this Example. To a suspension of pyrrole of Example 5 (5.83 g, 15.64 mmol) in dichloromethane (100 ml) is added benzoylisothiocyanate (2.88 g, 17.64 mmol) at room temperature under nitrogen. The reaction mixture is stirred for 30 min with the separation of the desired thioureido compound. Additional benzoyl isothiocyanate (0.5 ml) is added to it and again stirred for 30 min. The solvent is evaporated to dryness, and the light yellow residue is triturated with methanol. The white crystalline material is isolated by filtration and recrystallized from a chloroform-ether mixture to give the required thioureido compound as an analytically pure sample, yield 7.71 g (92%), mp 210-211 °C.

**EXAMPLE 7**

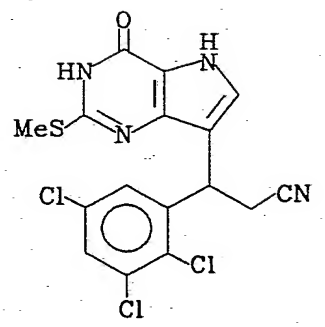


The above intermediate compound is prepared in this Example. A solution of thioureido compound of Example 6 (6.75 g, 12.6 mmol) and 1,5-diazabicyclo[4.3.0]non-5-ene (1.76 g, 14.20 mmol) in dry $CH_2Cl_2$ (200 ml) is cooled to 0 °C and treated with methyl iodide (5.20 g, 36.65 mmol). The reaction mixture is stirred at 0 °C for 10 min and then for 1 h at room temperature. The solvent is evaporated at room temperature, and the residue is extracted with $CHCl_3$, washed with $H_2O$ (2 x 50 ml), dried ($Na_2SO_4$) and evaporated to give a glassy foam (6.95 g) which is used in the next step without purification.

**EXAMPLE 8**



A



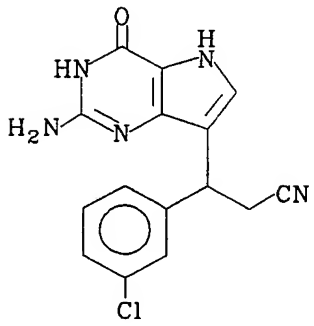
B

The above compounds A and B are prepared in this Example. The compound A is a compound of the present invention and the compound B is an intermediate. A solution of the methylthio intermediate of Example 7 (6.90 g, 12.54 mmol) in MeOH (200 ml) is saturated at 0 °C with ammonia and heated at 100 °C for 20 h in a glass-lined stainless steel bomb. The reaction mixture is brought to room temperature and the solvent is evaporated at room temperature. Purification of the crude mixture by flash column chromatography over silica gel using $CHCl_3$ as eluent gave **8B** (1.1 g, 21%), mp 290-291 °C then $CHCl_3$-MeOH (95:5) gave pure **8A** (2.76 g, 57.5%), mp 284-285 °C.

**EXAMPLE 9**

The compound of the present invention of Example 8 is tested for enzyme inhibition activity. A purine nucleoside phosphorylase (PNP) enzyme assay is performed in which the PNP activity ($IC_{50}$) for the compound (**8A**) is found, which is determined radiochemically by measuring the formation of [$^{14}$C]-hypoxanthine from [$^{14}$C]-inosine (see Biomedicine, **1980**, 33, 39) using calf spleen as the enzyme source. At 1 mM phosphate the $IC_{50}$ is 0.64 $\mu$M and at 50 mM phosphate the $IC_{50}$ is 10 $\mu$M.

**EXAMPLE 10**

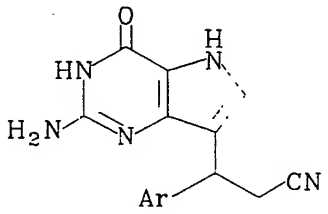


IC

Following the procedure set forth in Examples 1-8, 3-(3-chlorophenyl)-3-(2-amino-4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidin-7-yl)propanenitrile (IC) is prepared using 3-(3-chlorophenyl)-pentanedinitrile as the starting material, yield 54.5%, mp 157-158 °C.

**EXAMPLE 11**

Following the procedure set forth in Examples 1-8, the following compounds are also prepared (**1-9**).

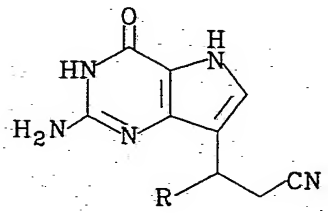


3-Aryl-3-(2-amino-4-oxo-3H,5H-pyrrolo[3,2-d]-pyrimidin-7-yl)propanenitrile

Where Ar is each of the following: (1) phenyl, 2,3-dichlorophenyl, 3-methylphenyl, and 3-methoxyphenyl, (2) thienyl (2- and 3-), (3) furanyl (2- and 3-), (4) pyridinyl (2-, 3-, and 4-), (5) pyrrolyl (2- and 3-), (6) thiazolyl (2-, 4-, and 5-), (7) 2-pyrazinyl, (8) pyridazinyl (3- and 4-), and (9) pyrazolyl.

**EXAMPLE 12**

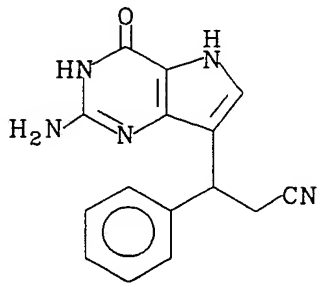
Following the procedure set forth in Examples 1-8, the following compounds 10-14 and 21 are prepared starting from the appropriately substituted pentanedinitrile. Compounds 15-20, and 22 are prepared from the corresponding unsaturated Ar analogues in Example 11. In this procedure, the nitrile group of the unsaturated analogue is first converted to an amide group by acid- or base-catalyzed hydrolysis, then the unsaturated Ar group is converted to the saturated $R^2$ group by known catalytic hydrogenation, followed by reconverting the amide back to the nitrile by known dehydration procedures.




3-(Substituted)-3-(2-amino-4-oxo-3H,5H-pyrrolo[3,2-d]-pyrimidin-7-yl)propanenitrile

Where $R^2$ is each of: 10) 1-adamantyl, 11) 2-adamantyl, 12) cyclohexyl, 13) cycloheptyl, 14) cyclopentyl, 15) tetrahydrofuranyl, 16) tetrahydrothienyl, 17) tetrahydropyranyl, 18) pyrazolidinyl, 19) thiazolidinyl, 20) piperazinyl, 21) morpholinyl, and 22) hexahydropyridazinyl.

**EXAMPLE 13**

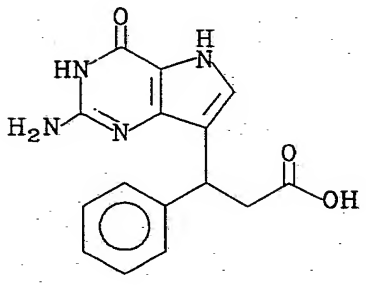


The above compound, 3-(2-amino-4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidin-7-yl)-3-phenylpropanenitrile, is prepared in this Example. A solution of the compound **A** obtained in Example 8 (2.0 g, 5.22 mmol) in warm ethanol (250 ml) and dimethylformamide (DMF) (150 ml) is hydrogenated over 30% Pd/C catalyst (1.0 g) in the presence of triethylamine (2.64 g, 5.0 equivalent) at atmospheric pressure. After 5 h, the reaction is complete, and the catalyst is filtered off under $N_2$ pressure. The solid obtained by evaporation of the filtrate is triturated and sonicated with $H_2O$ and dried, yield 1.28 g (88%), mp 168-170 °C.

**EXAMPLE 14**

The compound prepared in Example 13 is tested for enzyme inhibition activity as in Example 9. At 1 mM phosphate the $IC_{50}$ is 0.023 $\mu$M and at 50 mM phosphate the $IC_{50}$ is 4.7 $\mu$M.

**EXAMPLE 15**

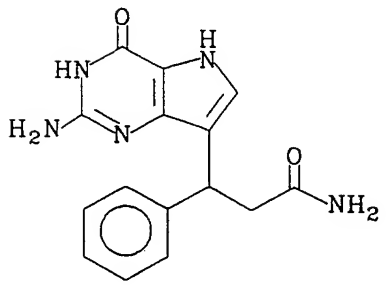


The above compound, 3-(2-amino-4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidin-7-yl)-3-phenylpropanoic acid, is prepared in this example. A solution of the compound obtained in Example 13 (0.200 g, 0.72 mmol) in 6N HCl (3.0 ml) is heated at reflux for 18 h. The solvent is evaporated in vacuo and the residue is triturated with $H_2O$ (6 ml), adjusted to pH ~10 by conc. ammonium hydroxide. Insoluble material is collected by filtration and the filtrate is readjusted to pH ~6.8. White material which is precipitated out is collected, washed with water and dried, yield 0.19 g (89%), mp 290 °C dec.

**EXAMPLE 16**

The compound prepared in Example 15 is tested for enzyme inhibition activity as in Example 9. At 1 mM phosphate the $IC_{50}$ is 0.012 $\mu$M and at 50 mM phosphate the $IC_{50}$ is 0.19 $\mu$M.

**EXAMPLE 17**

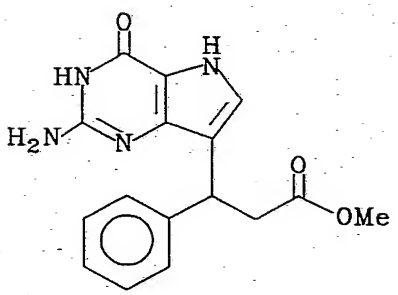


The above compound, 3-(2-amino-4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidin-7-yl)-3-phenylpropanamide, is prepared in this example. A solution of the compound obtained in Example 13 (0.200 g, 0.72 mmol) in conc. $H_2SO_4$ (0.5 ml) is stirred at room temperature for 20 h and then poured onto crushed ice (5.0 g) and adjusted to pH ~6.8 by conc. $NH_4OH$. The precipitated solid is collected, washed with $H_2O$ and dried, yield 0.180 g, mp 199-201 °C dec.

**EXAMPLE 18**

The compound prepared in Example 17 is tested for enzyme inhibition activity as in Example 9. At 1 mM phosphate the $IC_{50}$ is 0.20 μM and at 50 mM phosphate the $IC_{50}$ is 6.6 μM.

**EXAMPLE 19**

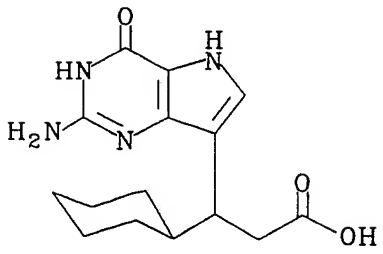


The above compound, 3-(2-amino-4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidin-7-yl)-3-phenylpropanoic acid, methyl ester, is prepared in this example. Thionyl chloride (0.2 g, 0.17 mmol) is added to stirred methanol (4.0 ml) at 0 °C. The compound obtained in Example 15 (0.2 g, 0.67 mmol) is added and the mixture is stirred at room temperature for 18 h. The solvent is removed on a water aspirator (30 °C) and vacuum pump (lyophilize) to give a semisolid mass which is purified on a silica gel column using $CHCl_3$-MeOH as the eluent, yield 0.1 g.

**EXAMPLE 20**

The compound prepared in Example 19 is tested for enzyme inhibition activity. Significant activity ($IC_{50}$) is found.

**EXAMPLE 21**



3-(2-Amino-4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidin-7-yl)-3-cyclohexylpropanoic acid is prepared in this example. A solution of the compound obtained in Example 15 (83 mg, 0.28 mmol) in trifluoroacetic acid (TFA) (15 ml) is hydrogenated with $PtO_2$ (83 mg) at 60 $lb/in^2$ for 24 h. The catalyst is filtered off through a Celite bed, and the filtrate is evaporated at 25 °C. The residue is suspended in $H_2O$ (8 ml), and adjusted to pH 8.5 by conc. $NH_4OH$ and filtered through a Whatman filter paper to remove brown colored impurities. The colorless filtrate is adjusted to pH ~6.8, and the precipitated compound is filtered, washed with $H_2O$, and dried, yield 65 mg (77%), mp >300 °C.

**EXAMPLE 22**

The compound prepared in Example 21 is tested for enzyme inhibition activity as in Example 9. At 1 mM phosphate the $IC_{50}$ is 0.097 $\mu$M and at 50 mM phosphate the $IC_{50}$ is 1.0 $\mu$M.

**EXAMPLE 23**

A compound of the present invention is prepared wherein X is $PO(OH)_2$. The nitrile group of the compound of Example 13 is converted to the corresponding amide by treatment with sulfuric acid. Using a Hoffman degradation reaction, the amide is converted to the corresponding amine, which is then converted to the corresponding pyridinium salt using a pyrillium salt. Conversion of the salt to the corresponding halide is accomplished using sodium bromide, which is then converted to the phosphonic ester using triethyl phosphite. Hydrolysis of the ester using trimethylsilylbromide yields the corresponding phosphonic acid wherein "n" is 1 and "m" is 0.

**EXAMPLE 24**

This Example makes a compound of the present invention by stepping up the number of carbon atoms from "m" is 0 to "m" is 1. The nitrile group of the compound of Example 13 is reduced to the corresponding aldehyde, which is then converted to the corresponding alcohol. Using phosphorous tribromide the alcohol is converted to the corresponding alkyl bromide, which is then converted to the nitrile compound of the present invention wherein m is 1 using potassium cyanide.

**EXAMPLE 25**

In this example a compound of the present invention is prepared wherein "p" is 1 and "Y" is oxygen. The alcohol prepared as an intermediate in the previous example is converted to the corresponding diethyl phosphonomethyl ether using

diethylchloromethyl phosphonate. Removal of the ethyl groups of the ester is accomplished using trimethylsilylbromide to give the phosphonic acid.

**EXAMPLE 26**

In this example a compound of the present invention is made wherein "Y" is NH and "X" is $SO_2NH_2$. The nitrile group of the compound of Example 13 is reduced to the amine using standard catalytic hydrogenation with palladium in acidic media (usually 0.01 N to 1 N HCl), which is then converted to the sulfamide using sulphamoyl chloride.

**EXAMPLE 27**

In this example a compound of the present invention is prepared wherein "X" is COOH and "Y" is NH by reacting the methyl amine intermediate prepared in the previous example with chloroacetic acid.

**EXAMPLE 28**

In this example a compound of the present invention is prepared wherein "X" is $PO(OH)_2$ and "Y" is NH by reacting the methyl amine intermediate prepared in Example 27 with diethylchloromethyl phosphonate, and reacting the resulting product with trimethylsilylbromide.

**EXAMPLE 29**

In this example a compound of the present invention is prepared wherein "X" is $SO_2NH_2$ and "Y" is oxygen by reacting the

alcohol intermediate prepared in Example 24 with sulphamoyl chloride.

**EXAMPLE 30**

In this example a compound of the present invention is prepared wherein $R^1$ is H, $R^2$ is phenyl, $R^3$ and $R^4$ are hydrogen, m is 0, n is 1, p is 0, and X is CN. A modification of the procedure disclosed in Mu-Ill Lim, et al., J. Org. Chem., Vol. 44, No. 22, 3826 (1979) is used. A mixture of the compound of Example 5 and dimethylformamide dimethyl acetal is reacted at room temperature for two days and then evaporated to dryness in vacuo. The residue is crystallized to give the pure N-(dimethylamino)methylene derivative, which is cyclized with saturated methanolic ammonia to give the desired end product.

**EXAMPLE 31**

In this example a compound of the present invention is prepared wherein $R^1$ is $OCH_3$, $R^2$ is phenyl, $R^3$ and $R^4$ are hydrogen, m is 0, n is 1, p is 0, and X is CN. Using the compound B of Example 8, the S-methyl group is oxidized to methylsulfone, which then is converted to the final methoxy compound by treatment with sodium methoxide in methanol.

**EXAMPLE 32**

In this example a compound of the present invention is prepared wherein X is tetrazole. The compound of Example 13 is treated with lithium azide in the presence of ammonium chloride

as a catalyst in dimethylformamide (DMF) at 100 degrees C to give the desired tetrazole.

**EXAMPLE 33**

In this example a compound of the present invention is prepared wherein X is triazole. The compound of Example 19 is treated with hydrazine hydrate to give the corresponding hydrazide, which is then treated with imino ether to give the desired triazole.

**EXAMPLE 34**

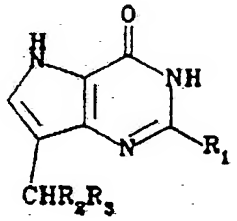
The compound prepared in Example 10 is tested for enzyme inhibition activity as in Example 9. At 1 mM phosphate the $IC_{50}$ is 0.012 $\mu$M and at 50 mM phosphate the $IC_{50}$ is 2.0 $\mu$M.

**EXAMPLE 35**

In this example an amidine compound of the present invention is prepared, i.e., wherein X in the recited generic formula is $CNHNH_2$. The compound A from Example 8 is reacted with sodium methoxide in methanol at room temperature for about 2 days to give a methyl-imidate intermediate. The intermediate is then reacted with ammonia in methanol to give the amidine product.

## EXAMPLES 36-42

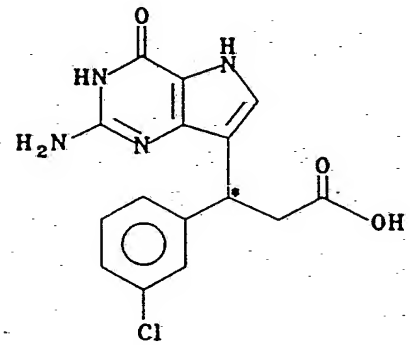
The following table gives the formulas for the compounds made in Examples 36-42 and the $IC_{50}$ (nM) values obtained for these compounds.



**Table**

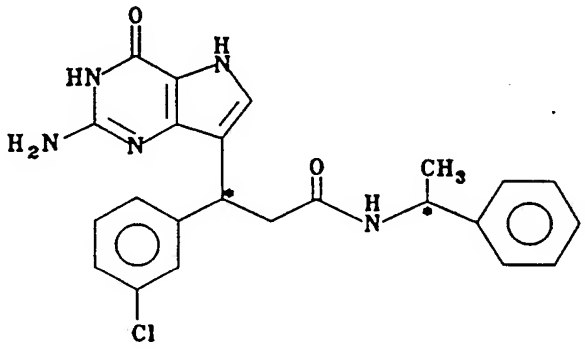
| No. | $R_1$ | $R_2$ | $R_3$ | $IC_{50}$ (nM) |
|---|---|---|---|---|
| Ex. 36 | $H_2N$ | 3-Chlorophenyl | $CH_2CO_2H$ | 7 |
| Ex. 37 | $H_2N$ | 3-Chlorophenyl | $CH_2CO_2H$ (S) | 5.9 |
| Ex. 38 | $H_2N$ | 3-Chlorophenyl | $CH_2CO_2H$ (R) | 160 |
| Ex. 39 | SMe | 3-Chlorophenyl | $CH_2CN$ | -- |
| Ex. 40 | H | 3-Chlorophenyl | $CH_2CN$ | 10 |
| Ex. 41 | $H_2N$ | 3-Chlorophenyl | $CH_2CH_2OH$ | 25 |
| Ex. 42 | $H_2N$ | 3-Chlorophenyl | $CH_2CO_2Me$ | 85 |
| 2,6-diamino-3,5-dihydro-7-(2-thienylmethyl)-4H-pyrollo-[3,2-d]pyrimidine-4-one (available from Warner-Lambert) | | | | 160 |

## EXAMPLE 36



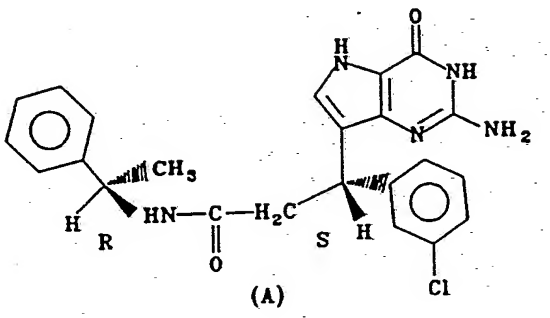
The compound prepared in Example 10 is hydrolyzed to the corresponding acid of the above formula in this example. A solution of 3-(3-chlorophenyl)-3-(2-amino-4-oxo-3H,5H-pyrrolo(3,2-d)pyrimidin-7-yl)propanenitrile (2.0 g; 63.75 mmol) in 6N HCl (60 ml) is heated at reflux for 8 h. The solvent is evaporated in vacuo and the residue is dissolved in $H_2O$ (18 ml). The resulting solution is adjusted to pH ~10 by conc. ammonium hydroxide and any insoluble material is removed by filtration. The filtrate is then readjusted to pH ~6.8. The white precipitated material was collected, washed with $H_2O$, and dried to yield 1.8 g of desired compound, m.p. 295-96°C dec, as a dl racemic mixture.

**EXAMPLE 37**

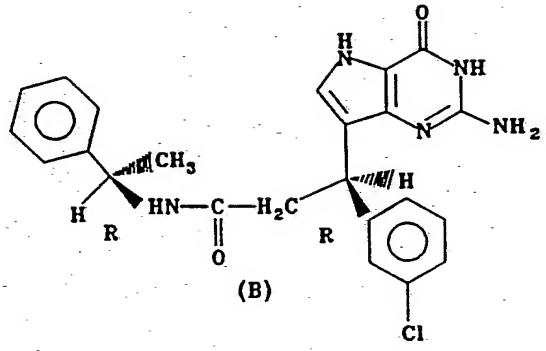


The above compound, 3-(2-amino-3H,5H-4-oxo-pyrrolo[3,2-d]pyrimidin-7-yl)-3-(3-chlorophenyl-N-(phenylethyl)propanamide, is prepared in this example. A solution of diphenylphosphoryl azide (0.72 g, 2.6 mmol) in DMF (10 ml) is added dropwise during 10 min to a mechanically-stirred, cold (-5 to 0°C) solution of the compound obtained in Example 36 (0.790 g; 2.4 mmol) and (R)d-(+)-α-methylbenzylamine

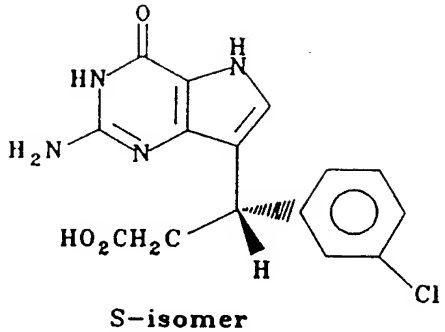
(0.32 g, 2.6 mmol) in DMF (100 ml). A solution of N-methylmorpholine (0.48 g, 4.75 mmol) in DMF (5 ml) is then added dropwise during 5-10 min, and the solution is kept near 0°C for 5 h. It is then allowed to warm to room temperature and is stirred overnight (18 h). A second portion of diphenylphosphonyl azide (0.36 g), (R)d-(+)-α-methylbenzylamine (0.16 g) and N-methylmorpholine (0.24 g) is added at 0°C and the reaction mixture is stirred for 2 days. The solvent is removed in vacuo and the residue is dissolved in an 8:2 mixture of acetonitrile and ammonium hydroxide (1M). The crude product is adsorbed on silica gel and dried in vacuo to remove the last traces of solvent. Flash column chromatographic purification using acetonitrile and 1M ammonium hydroxide (95:5) gives the pure desired material as a mixture of diastereomers (yield 0.630 g). These isomers are separated by repeated flash column chromatography on silica gel using acetonitrile and 1M ammonium hydroxide (98:2) as the eluent to yield 0.18 g of S,R-isomer (Compound A), m.p. 170-75°C dec. and 0.120 g of R,R-isomer (Compound B), m.p. 155-60°C dec.




(A)




(B)

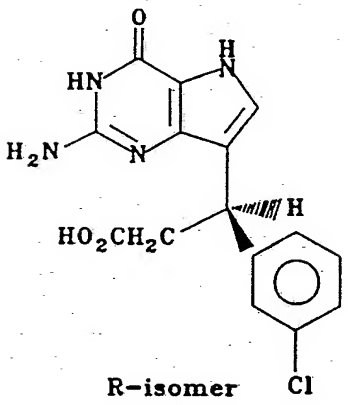


S-isomer

The above compound labeled "S-isomer," (S)-3-(2-amino-4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidin-7-yl)-3-(3-chlorophenyl)propanoic acid, is prepared in this example. A solution of the compound A (S,R-isomer) (0.170 g), obtained above, in 6N HCl (30 ml) is heated at reflux for 6 h and then left at room temperature for 6 h. The solvent is evaporated in vacuo and the residue is dissolved in $H_2O$ (5 ml). The resulting solution is adjusted to pH ~10 by conc. ammonium hydroxide and any insoluble material is removed by filtration. The filtrate is then readjusted to pH ~6.8 by ammonium hydroxide. The white precipitated material is collected, washed with $H_2O$, and dried to yield 0.090 g of the crude material which was purified by flash column chromatography using a 98:2 mixture of acetonitrile and ammonium hydroxide (1 M). Yield 48 mg, m.p. > 285°C dec.

A purine nucleoside phosphorylase (PNP) enzyme assay is performed in which the inhibitory activity ($IC_{50}$) of the S-isomer compound is determined by measuring the formation of [$^{14}$C]-hypoxanthine from [$^{14}$C]-inosine (see Biomedicine, 1980, **33**, 39) using calf spleen PNP in the presence and absence of inhibitor. At 50 mM phosphate the $IC_{50}$ is 0.031 $\mu$M and at 1 mM phosphate, it is 0.0059 $\mu$M.

**EXAMPLE 38**




The procedure described in Example 37 is repeated to prepare the above compound, (R)-3-(2-amino-4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidin-7-yl)-3-(3-chlorophenyl)propanoic acid from Compound B (R,R-isomer), obtained in Example 37. Yield 40%, m.p. > 280°C dec. The compound prepared in Example 38 is tested for enzyme inhibition activity as in Example 37. At 50 mM phosphate the $IC_{50}$ is 0.900 $\mu$M and at 1 mM phosphate the $IC_{50}$ is 0.160 $\mu$M. Thus the S-isomer (Example 38) is ca. 30X as potent as the R-isomer in the inhibition of PNP. X-ray crystallographic analysis of the enzyme-inhibitor complex formed from a soak of a crystal of the enzyme in a solution containing the unresolved racemic mixture (Example 36) showed that the S-isomer exclusively bound to the active site of the enzyme.

**EXAMPLE 39**

The procedures of Examples 1-8 are followed, except that the starting material used is the 3-chlorophenyl derivative rather than the 2,3,5-trichlorophenyl derivative used in the previous Examples. The SMe derivative as shown in the Table is obtained.

**EXAMPLE 40**

The compound from Example 39 (1 g) in ethanol (100 ml) is suspended in in 30% palladium on carbon (1 g) and subjected to reflux for a few minutes. Hydrazine hydrate (0.3 ml) is added with stirring an the mixture refluxed for two days. Additional hydrazine hydrate (0.3 ml) and palladium on carbon (0.5 g) are added and the mixture refluxed for an additional four days. The catalyst is removed by filtration, and the filtrate reduced to 25 ml and filtered on Whatman filter paper and evaporated to give the final product.

An alternative way of making the final product begins by using the present Examples 1-5 except that the 3-chlorophenyl derivative is used as the starting material. The resulting material, 3-amino-4-[(3-chlorophenyl)methyl]methylester-1-H-pyrolle-2-carboxylic acid, (5 g) is disolved in dimethylformamide dimethylacetal (50 ml) under argon and heated for 24 hours at 60-70 °C. After evaporation to dryness, the material is disolved in dichloromethane (50 ml), filtered and diluted with patroleum ether until cloudy, triturated to induce crystallization, and slowly diluted with and additional 40 ml of patroleum ether. This mono-chloro intermediate is collected, washed with patroleum ether and dried. Yield 5 g (88%), mp 122-124 °C. The resulting intermediate is heated in methanolic ammonia at 95-100 °C for 24 hours in a stainless steel bomb, evaporated to a yellowish solid crude product. The yellowish solid crude product (3 g) in 175 ml hot methanol yields a final product of 2.2 g (88% yield).

**EXAMPLE 41**

The compound of Example 10 (6.80 g) in 6N HCl (400 ml) is refluxed for 10 h, cooled overnight, and evaporated under reduced pressure. The residue is added to methanol and evaporated and then added to toluene, which results in a white foam in nearly quantitative yield. A solution of the dried white foam in anhydrous methanol (400 ml) is cooled below 0°C in an ice salt bath under dry conditions. Thienyl chloride (10.31 g) is added slowly dropwise, and the solution allowed to come to ambient temperature and stand overnight. The solvent is evaporated in vacuo, fresh methanol and toluene are added and then evaporated to aid in the removal of acid vapors. A suspension of the solid in cold water (200 ml) is neutralized in 1N NaOH and the solid is collected by filtration, washed with cold water, and dried in vacuo over $P_2O_5$ at 110°C. Yield 6.08 g (81% from the material of Example 10). This product is of sufficient purity for use in the next step, but may recrystallize in methanol using Soxhlet apparatus to fine white crystals having a m.p. of 302-303°C (decompose). An amount of 6g of the product from the previous paragraph with 100 mg dry ammonium sulfate in hexamethyldisilazane (400 ml) is refluxed for 8 h under dry conditions. The resulting clear solution is evaporated in vacuo to a viscous gum that is further dried over $P_2O_5$, which is used in the next Example without further treatment.

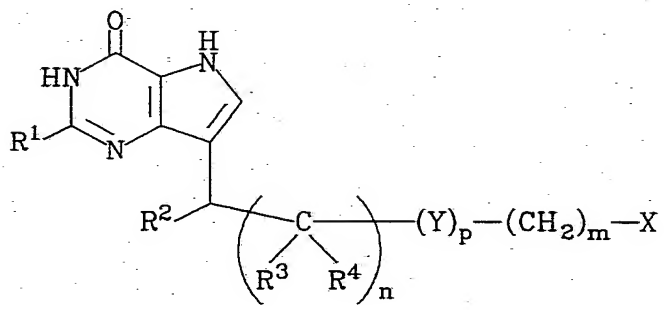
**EXAMPLE 42**

Under nitrogen, a solution of the product from the previous paragraph in anhydrous THF or ether (200 ml) is treated dropwise

with a 1 molar solution of lithium aluminum hydride (26 ml) in THF. After 1 h at room temperature, excess hydride is destroyed by dropwise addition of ethyl acetate (50 ml), and the solvent evaporated in vacuo. The residue is suspended in cold water (200 ml), adjusted to a pH of 1 with HCl, stirred for 15 min, adjusted to a pH 7 with dilute sodium hydroxide, and filtered. The resulting filter cake is washed with cold water, dried, and washed with ethyl to remove TMS by-products. Silica gel (50 g) is added to a hot solution of the resulting crude solid (~ 8 g) in a large volume of methanol, and the resulting slurry is evaporated to dryness. The resulting material is layered carefully onto a silica gel column that is eluted with a chloroform/methanol mixture (85/15) to give the desired alcohol final product. Yield 4.65 g (84%). Two recrystallizations from ethanol/water gives a white crystalline material. Yield 3.36 g (61%), m.p. 255-277°C (decompose).

**CLAIMED IS:**

1. A PNP inhibitor comprising a compound of the formula



wherein $R^1$ is H, $NH_2$, or $OCH_3$, $R^2$ is an optionally substituted cyclic group optionally containing one or more heteroatoms, $R^3$ and $R^4$ are independently H or $C_{1-4}$ alkyl, m is 0-4, n is 0-6, p is 0-1, X is CN, $CSNH_2$, $PO(OH)_2$, COOH, $SO_2NH_2$, $NH_2$, OH, $CNHNH_2$, tetrazole, or triazole, $COR^5$ where $R^5$ is $C_{1-4}$ alkyl, $CF_3$, $NH_2$, or $OC_{1-4}$ alkyl, and Y is O or NH.

2. The inhibitor of claim 1 wherein $R^2$ is unsubstituted.

3. The inhibitor of claim 2 wherein $R^1$ is $NH_2$, $R^3$ and $R^4$ are H, m is 0 and n is 1.

4. The inhibitor of claim 3 wherein $R^2$ is phenyl.

5. The inhibitor of claim 4 wherein X is CN.

6. The inhibitor of claim 4 wherein X is COOH.

7. The inhibitor of claim 4 wherein X is $CONH_2$.

8. The inhibitor of claim 3 wherein $R^2$ is 2- or 3-thienyl, 2-or 3-furanyl, 2-, 3-, or 4-pyridinyl, 2- or 3-pyrrolyl, 2-, 4-, or 5-thiazolyl, 2- or 3-pyrazinyl, 3- or 4-pyridazinyl, or pyrazolyl.

9. The inhibitor of claim 8 wherein X is CN, COOH, or $CONH_2$.

10. The inhibitor of claim 3 wherein $R^2$ is 1- or 2-adamantyl, cyclopentyl, cyclohexyl, cycloheptyl, 2- or 3-tetrahydrofuranyl, 2- or 3-tetrahydrothienyl, 2- or 3-tetrahydropyranyl, 2-, 3-, or 4-piperidinyl, 3- or 4-pyrazolidinyl, 2-, 4-, or 5-thiazolidinyl, 2- or 3-piperazinyl, 2- or 3-morpholinyl, or hexahydropyridazinyl.

11. The inhibitor of claim 10 wherein X is CN, COOH, or $CONH_2$.

12. The inhibitor of claim 3 wherein $R^2$ is cyclohexyl and X is COOH.

13. The inhibitor of claim 1 wherein $R^2$ is an optionally substituted 5- or 6-membered aromatic or heteroaromatic group.

14. The inhibitor of claim 1 wherein $R^2$ is an optionally substituted alicyclic group or heteroalicyclic group of 5-9 members.

15. The inhibitor of claim 1 wherein $R^2$ is substituted with at least one of halogen, hydroxy, $C_{1-4}$ alkoxy, $C_{1-4}$ alkyl, or trifluoromethyl.

16. A method for the selective suppression of mammalian T-cell function without diminished effect on humoral immunity comprising administering to a subject an effective amount of the PNP inhibitor of claim 1.

17. A method for the selective suppression of mammalian T-cell function without diminished effect on humoral immunity comprising administering to a subject an effective amount of the PNP inhibitor of claim 5.

18. A method for the selective suppression of mammalian T-cell function without diminished effect on humoral immunity

comprising administering to a subject an effective amount of the PNP inhibitor of claim 6.

19. A method for the selective suppression of mammalian T-cell function without diminished effect on humoral immunity comprising administering to a subject an effective amount of the PNP inhibitor of claim 7.

20. A method for the selective suppression of mammalian T-cell function without diminished effect on humoral immunity comprising administering to a subject an effective amount of the PNP inhibitor of claim 8.

21. A method for the selective suppression of mammalian T-cell function without diminished effect on humoral immunity comprising administering to a subject an effective amount of the PNP inhibitor of claim 10.

22. A method for the selective suppression of mammalian T-cell function without diminished effect on humoral immunity comprising administering to a subject an effective amount of the PNP inhibitor of claim 12.

23. A pharmaceutical composition for the selective suppression of mammalian T-cell function without diminished effect on humoral immunity comprising an effective amount of the PNP inhibitor of claim 1 and a pharmaceutically acceptable carrier or diluent.

24. A pharmaceutical composition for the selective suppression of mammalian T-cell function without diminished effect on humoral immunity comprising an effective amount of the PNP inhibitor of claim 5 and a pharmaceutically acceptable carrier or diluent.

25. A pharmaceutical composition for the selective suppression of mammalian T-cell function without diminished effect on humoral immunity comprising an effective amount of the PNP inhibitor of claim 6 and a pharmaceutically acceptable carrier or diluent.

26. A pharmaceutical composition for the selective suppression of mammalian T-cell function without diminished effect on humoral immunity comprising an effective amount of the PNP inhibitor of claim 7 and a pharmaceutically acceptable carrier or diluent.

27. A pharmaceutical composition for the selective suppression of mammalian T-cell function without diminished effect on humoral immunity comprising an effective amount of the PNP inhibitor of claim 8 and a pharmaceutically acceptable carrier or diluent.

28. A pharmaceutical composition for the selective suppression of mammalian T-cell function without diminished effect on humoral immunity comprising an effective amount of the PNP inhibitor of claim 10 and a pharmaceutically acceptable carrier or diluent.

29. A pharmaceutical composition for the selective suppression of mammalian T-cell function without diminished effect on humoral immunity comprising an effective amount of the PNP inhibitor of claim 12 and a pharmaceutically acceptable carrier or diluent.

30. A method for making a chemical compound comprising the steps of:

a) reacting an optionally substituted cyclic aldehyde with cyanoacetic acid in the presence of ammonium acetate to make a 3-cyclo-substituted pentanedinitrile;

b) reacting the 3-cyclo-pentanedinitrile with an alkyl formate and a base to make a 3-cyclo-2-formylpentanedinitrile;

c) reacting the 3-cyclo-2-formylpentanedinitrile with glycine methyl ester hydrochloride and sodium or ammonium acetate to make methyl N-[(3-cyclo-2,4-dicyano)-2-butenyl]glycine;

d) reacting the methyl N-[(3-cyclo-2,4-dicyano)-2-butenyl]glycine with an alkyl chloroformate and DBN or DBU to make methyl 3-amino-4-(2-cyano-1-cyclo-ethyl)-1-ethyl-1H-pyrrole-1,2-dicarboxylate; and

e) reacting the methyl 3-amino-4-(2-cyano-1-cyclo-ethyl)-1-ethyl-1H-pyrrole-1,2-dicarboxylate with a base to make methyl 3-amino-4-(2-cyano-1-cyclo-ethyl)-1H-pyrrole-2-carboxylate.

31. The method of claim 30 further comprising the steps of:

f) reacting the methyl 3-amino-4-(2-cyano-1-cyclo-ethyl)-1H-pyrrole-2-carboxylate with benzoylisothiocyanate to make N-benzoyl-N'-[4-(2-cyano-1-cyclo-ethyl)-2-methoxycarbonyl-1H-pyrrol-3-yl]thiourea;

g) reacting N-benzoyl-N'-[4-(2-cyano-1-cyclo-ethyl)-2-methoxycarbonyl-1H-pyrrol-3-yl]thiourea with an alkyl halide to make N-benzoyl-N'-[4-(2-cyano-1-cyclo-ethyl)-2-methoxycarbonyl-1H-pyrrol-3-yl]S-methylthiourea; and

h) reacting N-benzoyl-N'-[4-(2-cyano-1-cyclo-ethyl)-2-methoxycarbonyl-1H-pyrrol-3-yl]-S-methylthiourea with methanolic

or ethanolic ammonia to make a mixture of 3-cyclo-3-[2-amino-4-oxo-3H-5H-pyrrolo[3,2-d]pyrimidin-7-yl]propanenitrile and 3-cyclo-3-[2-methylmercapto-4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidin-7-yl]propanenitrile.

32. The method of claim 31 further comprising the steps of:

i) reacting the 3-cyclo-3-[2-methylmercapto-4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidin-7-yl]propanenitrile with an oxidizing agent to make 3-cyclo-3-[2-methylsulfonyl-4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidin-7-yl]propanenitrile; and

j) reacting the 3-cyclo-3-[2-methylsulfonyl-4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidin-7-yl]propanenitrile with sodium alkoxide to make 3-cyclo-3-[2-methoxy-4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidin-7-yl]propanenitrile.

33. The method of claim 30 further comprising the steps of:

f) reacting the methyl 3-amino-4-(2-cyano-1-cyclo-ethyl)-1H-pyrrole-2-carboxylate with dimethylformamide dimethyl acetal to make methyl 4-(2-cyano-1-cyclo-ethyl)-3-[N-(dimethylaminomethylene)amino]-1H-pyrrole-2-carboxylate; and

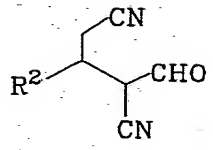
g) reacting the methyl 4-(2-cyano-1-cyclo-ethyl)-3-[N-(dimethylaminomethylene)amino]-1H-pyrrole-2-carboxylate with methanolic ammonia to make 3-cyclo-3-[4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidin-7-yl]propanenitrile.

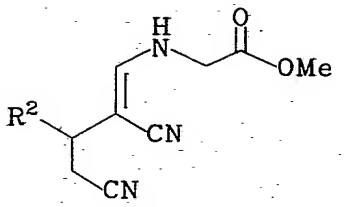
34. The method of claim 30 wherein the cyclic substituent is phenyl.

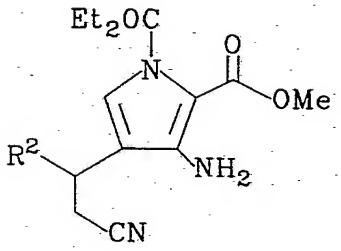
35. The method of claim 30 wherein the cyclic substituent is 2- or 3-thienyl, 2- or 3-furanyl, 2-, 3-, or 4-pyridinyl, 2- or 3-pyrrolyl, 2-, 4-, or 5-thiazolyl, 2-or 3-pyrazinyl, 3- or 4-pyridazinyl, or 3-, 4-, or 5-pyrazolyl.

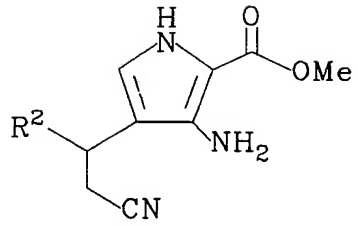
36. The method of claim 30 wherein the cyclic substituent is 1- or 2-adamantyl, cyclopentyl, cyclohexyl, cycloheptyl, and morpholinyl.

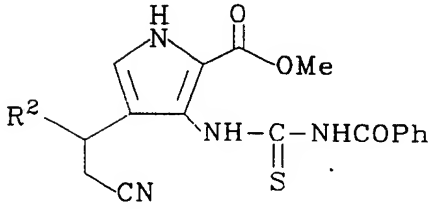
37. A compound of the formula

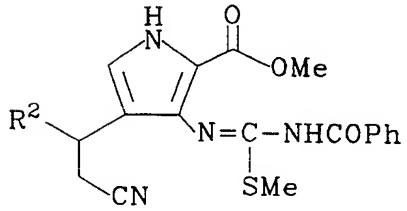


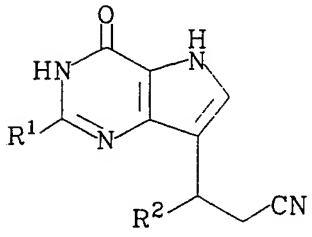



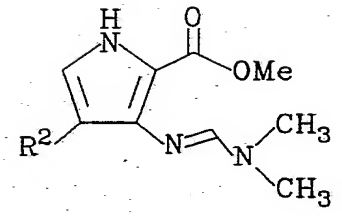





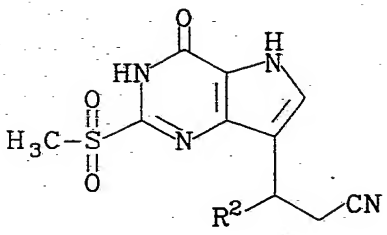








or



wherein $R^1$ and $R^2$ are as defined claim 1.

# INTERNATIONAL SEARCH REPORT

:rnational application No.
PCT/US93/02841

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(5) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C.A.S. online, structure searches

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO 91/06548 (Biocryst) 16 May 1991. See claims 5-16. | 1-37 |
| P,X | US, A 5189039 (Niwas) 23 Feb. 1993. See Ex. 1-7 and Claims 1 and 19. | 1-37 |
| X | Pro. Natl. Acad. Sci. Vol. 88 (Dec. 1991) p. 11540-11544. Ealick et al. "Application of crystallographic and modeling methods in the design of purine nucleoside phosphorylase inhibitors". See Table 1, first, second and next to last species. | 1, 13, 16, 23, 37 |

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 10 AUGUST 1993 | 19 AUG 1993 |
| Name and mailing address of the ISA/US<br>Commissioner of Patents and Trademarks<br>Box PCT<br>Washington, D.C. 20231<br>Facsimile No. NOT APPLICABLE | Authorized officer<br>MARK L. BERCH<br>Telephone No. (703) 308-1235 |

Form PCT/ISA/210 (second sheet)(July 1992)★

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/02841

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:
(Form PCT/ISA/206 Previously Mailed.)
Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

☒ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/02841

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (5):

CO7D 487/04, 265/30, 237/08, 241/12, 213/57, 233/64, 277/30 207/337, 207/34, 333/24, 307/81; CO7C 255/42, 255/40, 255/31; CO7F 9/38; A61K 31/505, 31/535

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

514/258, 234.2, 253,81, 212; 544/224, 232, 117, 141, 163, 238, 280, 336, 405; 548/204, 374, 378, 519, 532, 533, 561; 546/281, 330; 549/76, 77, 494; 558/404, 406, 429, 430, 432

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

514/258, 234.2, 253,81, 212; 544/224, 232, 117, 141, 163, 238, 280, 336, 405; 548/204, 374, 378, 519, 532, 533, 561; 546/281, 330; 549/76, 77, 494; 558/404, 406, 429, 430, 432

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

See attached.

I. Claims 1-3 (part), 4-7, 10-11 (part), 12, 13-16 (part), 17-19, 22, 24-26, 29, 34 (part), drawn to $R_2$=Non-Hetero.

II. Claims 1-3 (part), 8-9, 10-11 (part), 20, 21, 25, 27, 34 (part), drawn to $R_2$=Hetero.

III. Claims 30, 34-36, drawn to Process #1.

IV. Claim 31, drawn to Process #2.

V. Claim 32, drawn to Process #3.

VI. Claim 33, drawn to Process #4.

VII. Claim 37 (part), drawn to Aldehyde Intermediates.

VIII. Claim 37 (part), drawn to Unsaturated Nitrile Intermediates.

IX. Claim 37 (part), drawn to Pyrrole Intermediates.

X. Claim 37 (part), drawn to Pyrrolopyrimidine Intermediates.

and it considers that the international application does not comply with the requirements of unity of invention (Rules 13.1, 13.2 and 13.3) for the reasons indicated below:

Groups I and II are distinct from each other because of the presence (in Group II) and absence (in Group I) of the heterocyclic group in $R_2$. Hetero and non-hetero rings are normally considered to be patentably distinct variants.

The various processes are considered to be distinct from each other because they involve different kinds of chemical reactions. Group III involves the synthesis of a pyrrole ring Group IV involves the preparation and then transformation of a thiourea. Group V is an oxidative process; Group VI involves an acetal condensation. Further, the steps are not sequential, in that, for example, the step (f) of Group IV (or VI for the matter) does not begin with the product of step (e). The processes are different from the Group I-II materials, in that the processes do not make e.g. the p=1 products, or the X=$CO_2H$ materials.

In terms of Claim 37, Group VII corresponds to the first appearing structure formula, Group VIII, the second, Group IX all the remainder except for the last. These groups are clearly structurally different due to the marked structural differences present. The Group VII compounds, for example, bear very little structural resemblance to the

Group VIII materials.